Studies on Cucumber Softening under Commercial Salt-Stock Conditions in Ontario[1]

II. Pectolytic Microorganisms Isolated

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The investigation of cucumber softening can be traced back to the turn of the century when Aderhold (1899) described a sporeforming organism, which he called *Bacterium coli*, as the cause of this spoilage. Kossowicz (1908), however, implicated the genus *Bacillus* and further work by Rahn (1913), LeFevre (1919), and Joslyn (1929) confirmed and extended this theory. Fabian and Johnson (1938) continued the study of the aerobic, sporeforming, mesophilic bacteria and were successful in isolating *Bacillus mesentericus fuscus* (*Bacillus subtilis*) from brine containing soft cucumbers. It was shown that this organism grew readily in 9 per cent salt, 0.2 per cent acetic acid, and 0.3 per cent lactic acid. In addition, it was shown that the cultural filtrate of this organism, after growth in beet molasses medium, caused spoilage of firm desalted cucumbers in 24 hr.

Despite the evidence implicating the genus *Bacillus*, it is generally considered that this group probably plays a very minor role in softening under normal curing conditions. Fabian and Johnson (1938) were probably the first to cast doubt on this theory when they found the pectic enzymes of *B. subtilis* were inhibited by 2 per cent salt. They were able to increase the salt tolerance of the enzymes to 7 per cent salt by continuous culture at high concentrations of salt, but as softening occurs at much higher levels, it was concluded that spoilage by this organism was unlikely.

More recently Nortje and Vaughn (1953) carried out qualitative studies with *B. subtilis* and *Bacillus pumilis* in relation to cucumber softening and indicated further doubt as to the importance of bacterial spore-formers as causal agents. The pectolytic enzyme complex of *B. subtilis*, similar to polygalacturonase, was found to be most active at pH 8.6 and reasonably stable in an alkaline environment, but lost its activity under acid conditions.

Vaughn *et al.* (1954) concluded that species of *Bacillus* would carry out softening of brined cucumbers only if they predominated the microbial populations of the salt-stock brines, if the pH of the brine remained 5.5 or above, and if the lactic acid fermentation was retarded such that the total acidity remained relatively low for several days.

The incidence of yeast flora in commercial cucumber salt-stock has been studied extensively by Etchells and co-workers (1941, 1950, 1953). Etchells and Bell (1950) found that of the surface yeasts isolated from salt-stock brines, *Debaromyces*, *Endomycopsis*, and *Candida* species de-esterified pectin. Luh and Phaff (1951) found generally that few yeasts were active against liquid pectin. Of the large number of yeasts studied, only *Saccharomyces fragilis* and varieties of this species were found active. In a more recent study, Bell and Etchells (1956) found that in addition to the surface yeasts de-esterifying pectin, species of *Hansenula*, *Rhodotorula*, and *Zygopichia* were also capable of elaborating pectinesterase. However, none of the yeasts isolated from brines was found to carry out glycosidic hydrolysis as compared to nonbrine species of *S. fragilis*.

Etchells, Bell, and Jones (1955) implicated fungi as the causal agents of cucumber softening. These workers contended that fungi occurred in salt-stock fermentations as contaminants on cucumber flowers which remained attached to the fruit after the blooming period. It was noted that brine from vats filled with a high incidence of flowers exhibited increased pectolytic activity compared to brine from tanks where the flowers had been removed prior to fermentation.

Etchells *et al.* (1958) continued the study in relation to cucumber flowers and fruit. High fungal populations were noted from both cucumber flowers collected in the field and at the commercial brining stations. It was again noted that the pectolytic activity of brined, flower-laden cucumbers was higher than that for the flowerless fruit. Of the 72 species of fungi isolated in this work, the majority proved to be both pectinolytic and cellulolytic. Five genera, *Penicillium*, *Ascochyta*, *Fusarium*, *Cladosporium*, and *Alternaria*, represented 60 per cent of all the isolates and were found to be important sources of pectinolytic enzyme activity. The authors

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concluded that filamentous fungi were definitely implicated in softening under commercial conditions found in the southern United States.

During the 1958 to 1959 brining seasons, a microbiological study was carried out in connection with the incidence and pattern of activity of pectolytic enzymes in commercial cucumber salt-stock brines in Ontario (Hamilton and Johnston, 1961). This study included the isolation, from commercial brines, of organisms which elaborated pectolytic enzymes on a laboratory medium. Additional tests were carried out in an endeavor to determine which of the types of organisms: bacteria, yeasts, or filamentous fungi, have the ability to produce pectolytic enzymes under the environmental conditions of commercial vats.

A number of important prerequisites must be satisfied by an organism before it can be considered a causal agent of pectolytic softening under commercial conditions. Such an organism must have the ability to (a) withstand the salt and acid concentrations of a cucumber salt-stock brine, (b) produce pectolytic enzymes under these conditions, and (c) elaborate these enzymes in high concentrations for an extended period.

To test the organisms isolated for the above prerequisites, a series of experiments were proposed simulating vat conditions and testing for the presence of the enzymes resembling the polygalacturonases, the polymethylgalacturonases and pectinesterase (Demain and Phaff, 1957).

Materials and Methods

Isolation

Media and procedures—1958. During the commercial brining season of 1958, pectolytic organisms from commercial salt-stock vats were isolated from some 56 vats in Guelph and Chatham, Ontario. The sodium polypectate medium proposed by Vaughn *et al.* (1957) for the isolation of pectolytic organisms was used at three pH levels: pH 6.5, for the isolation of bacteria; pH 4.2, for the isolation of yeasts; and pH 3.3, for the isolation of filamentous fungi.

Following the preparation and subsequent sterilization of this medium, it was allowed to cool to room temperature before pouring into Petri plates and then allowed to solidify for several hours. These plates were stored at 5 C until they were required, with care being taken to dry them before use.

In addition to the poured sodium polypectate gel plates, deep test tubes of the same medium were prepared at pH 4.2 for the isolation of anaerobic organisms showing pectolytic activity.

During the first 12 days of sampling, the sodium polypectate gel (SPG) at pH 6.5, 4.2, and 3.3 along with the anaerobic tubes at pH 4.2, were employed for isolation. However, from the 14th day and including the 18th, 22nd, and 68th days, all isolations were made from the SPG adjusted to pH 3.8. The pH of the brine was rarely found to exceed this level at this stage of fermentation.

Each brine sample was swabbed onto plates of solidified sodium polypectate gel immediately upon arrival at the laboratory. Following this streaking, the plates were incubated at 30 C for 3 to 5 days and then examined for any evidence of liquefaction. If liquefaction occurred, the organism or organisms responsible were then streaked on slants of beef extract agar containing 6 per cent sodium chloride. The slants were incubated at 30 C until growth appeared followed by storage at 5 C until purification was possible.

Media and procedures—1959. In an effort to contribute to the current knowledge concerning the fungal populations in the salt-stock brines, a brief survey was conducted during the 1959 brining season to test for the presence of these organisms in the brine. The method involved the streaking of the initial or 1st day samples of each test vat on Littman's (1947) medium with a 0.01-ml wire loop in order to obtain an approximate count present in the brine at the time of sampling. Littman's medium included streptomycin (30 mg per ml) to inhibit bacterial growth. Incubation was carried out at 25 C. Fungal cultures isolated were streaked on potato dextrose agar slants and stored at 5 C until purification could be carried out.

All of the microbial cultures obtained during the two periods were purified and streaked on sodium polypectate gel and retained if they liquefied the medium. The cultures obtained from the deep stab tubes were handled like the bacterial isolates with the exception that the streaked sodium polypectate plates were incubated aerobically and anaerobically under an atmosphere of hydrogen. All of the fungi isolated were identified, as were a number of the bacterial cultures.

Brine Studies with Pure Cultures

Brine medium. Large quantities of commercial cucumber salt-stock brine at pH 3.4 and 16 per cent salt, were dialyzed for various periods to lower the salt concentration and the resulting solutions adjusted to four separate salt and pH levels: test I, pH 5.3 and 6.0 per cent salt; test II, pH 4.0 and 9.0 per cent salt; test III, pH 3.4 and 12.8 per cent salt; and test IV, pH 5.3 and no salt. In all cases, pectin (8.0 per cent methoxyl) was added to the brine medium to a final concentration of 0.5 per cent to act as a substitute for cucumber protopectin.

Test I represented the conditions occurring during the 1st to 3rd days of fermentation, whereas test II represented the conditions between the 5th and 9th day when the polygalacturonase activity of the brine usually

began to decline. Test III was representative of the conditions during the latter stages of curing when the enzyme activities were observed to be very low. Test IV served as a control. In addition, all organisms used in the tests were also streaked on sodium polypectate gel to reaffirm their ability to produce an enzyme similar to polygalacturonase on laboratory medium.

The brine medium was distributed to 2-ounce bottles in 25-ml amounts, capped, and sterilized. The control series was sterilized for 15 min at 15 lb and the other groups were sterilized at 10 lb for 10 min. In each case, the medium was cooled immediately following the sterilization to prevent further denaturation of the pectin.

Fifteen yeasts and 141 bacterial cultures were randomly selected from the isolates and separated into groups according to their degree of hydrolysis of sodium polypectate gel. Fifty-six fungal cultures were utilized for this work and were similarly grouped. Included with the pectolytic bacteria was a group of 51 nonpectolytic organisms to act as controls.

Procedure. Twenty-four hours prior to the incubation of the brine medium, the bacterial and yeast cultures were transferred to test tubes containing 5 ml of beef extract broth +0.1 per cent glucose and incubated at their optimal temperatures. For inoculation of the brine medium, the resulting broth suspension was adjusted to a constant turbidity by dilution with sterile broth. One milliliter of this suspension was then transferred to the brine medium, mixed, and incubated at 24 C for 3 days.

Filamentous fungi were prepared for inoculation by adding 2 or 3 ml of broth to a culture slant and washing the mycelium and spores into suspension with a sterile needle. To the brine medium, 0.5 ml of this suspension was added, followed by thorough mixing, and incubation at 24 C for 3 days. All fungal tests were carried out in duplicate because of the difficulty in estimating the amount of growth added to the brine.

Following the incubation period, all the samples were dialyzed in demineralized water at 5 C for 5 hr. Prior to this dialysis, all samples containing fungi were filtered to remove the mycelial growth and the majority of spores. After the dialysis, the samples were removed to test tubes containing 0.5 ml of toluene to prevent further growth, and these tubes were placed in a 30 C water bath for equilibration prior to the enzyme tests.

Enzyme Tests

Polygalacturonases. The method of Bell *et al.* (1955) was used for the polygalacturonases (PG) test with the exception that a 0.9 per cent solution of sodium polypectate adjusted to pH 5.3 was used in this case. The same substrate was used in all four series. The tests were made at 30 C and incubation carried out at the same temperature with the final readings being made at 20 hr.

Pectinesterase. The titration method of pectinesterase (PE) assay (Hamilton and Johnston, 1961) was used in this work, with the exception, however, that the incubation period was extended to 68 hr at 30 C. Five drops of toluene were included in the reaction mixture to prevent microbial growth.

Polymethylgalacturonases. This group of enzymes (PMG) is specific for the substrate pectin according to the classification of Demain and Phaff (1957). Therefore, a viscosity method similar to that of Bell *et al.* (1955) was used by substituting pectin for the sodium polypectate substrate. A 1.3 per cent solution of pectin (8.0 per cent methoxyl), buffered by sodium hydroxide and citric acid at pH 5.3, was used in Ostwald-Fenske pipettes and gave flow-times similar to a 0.9 per cent solution of sodium polypectate.

The procedure involved added 15 ml of the pectin solution and 5 drops of toluene to a 1-ounce sterile bottle. This solution was then allowed to equilibrate at 30 C for 30 min. At 0 time, 3 ml of the dialyzed enzyme sample were added to the bottle and the solution mixed thoroughly. Six milliliters of this reaction mixture were then immediately removed to an equilibrated viscometer and the initial flow time recorded. The bottles containing the reaction solution were then incubated at 30 C for 68 hr, at which time 6 ml of the solution were again removed to the same viscometer and the final reading observed. The same calculations were used here for the loss in viscosity of the pectin solution as were employed for the polygalacturonase viscosity test (Bell *et al.*, 1955).

Control samples were included with each of the daily tests with the appropriate corrections made to all the calculations for the changes observed in the control readings.

RESULTS

A total of 429 pure, pectolytic cultures was obtained for the 56 commercial vats tested in 1958. Of this total, 393 were found to be gram positive, sporeforming bacteria resembling the genus *Bacillus*, 21 were yeasts, and only 15 were cultures of filamentous fungi.

In 1959, only 42 cultures of filamentous fungi were obtained from 90 commercial vats. All but one of these cultures were found to be pectolytic on sodium polypectate medium upon purification. The total of 56 pectolytic fungi from both sampling seasons were identified and are presented in table 1. In addition to the filamentous fungi isolated, some 17 cultures of yeasts were also obtained but not identified. Of the yeast cultures obtained, only 15 were found to be pectolytic and to a very slight degree.

The cultures isolated from the deep tubes of the sodium polypectate gel included facultative anaerobes, while the remainder were found to be aerobic. None of these cultures was found to be pectolytic.

TABLE 1

Pectolytic enzymes elaborated by filamentous fungi, bacteria, and yeasts under simulated commercial salt-stock conditions at four pH and sodium chloride levels

Organism	Number Tested	Action on SPG*	Polygalacturonase†				Polymethylgalacturonase‡				Pectinesterase§			
			Percent loss in viscosity				Percent loss in viscosity				Mg methoxyl per ml			
			Test I	Test II	Test III	Test IV	Test I	Test II	Test III	Test IV	Test I	Test II	Test III	Test IV
FUNGI														
Alternaria														
A. fasciculata	1	3+¶	46.2‖	20.5	1.0	—**	56.7	21.0	3.5	—	0.0	0.0	0.0	—
A. humicola	7	3+	31.7	20.3	13.1	69.4	38.0	9.5	7.7	46.4	0.0	0.0	0.0	0.0
A. tenuis	1	3+	22.6	2.0	1.7	—	22.6	4.4	1.3	—	0.0	0.0	0.0	—
Aspergillus														
A. flavus	1	3+	8.2	17.6	1.0	29.4	30.2	26.1	4.2	56.3	0.0	0.0	0.0	0.5
A. fumigatus	1	3+	48.0	11.0	1.0	—	43.5	27.6	3.6	—	0.0	0.0	0.0	—
A. niger	1	4+	81.8	88.7	93.5	—	45.6	86.5	80.0	—	0.0	0.0	0.2	—
A. sydowi	2	2+	17.4	36.0	30.4	—	9.8	21.5	19.0	—	0.0	0.0	0.0	—
Cephalosporium														
C. acremonium	2	2+	10.3	6.3	4.0	29.6	7.7	6.0	3.0	40.8	0.0	0.0	0.0	0.0
C. coremioides	6	2+	3.3	6.2	2.5	—	10.1	4.2	3.6	—	0.0	0.0	0.0	—
C. roseo-griseum	5	3+	35.3	35.6	2.0	—	57.7	32.6	4.5	—	0.0	0.0	0.0	—
Epicoccum														
E. nigrum	1	2+	22.4	2.9	1.0	94.4	17.3	2.5	7.0	83.9	0.0	0.0	0.0	0.4
Fusarium														
F. concolor	1	2+	3.0	5.0	1.7	91.3	10.2	4.4	5.2	76.7	0.0	0.0	0.0	0.0
F. culmorum	1	2+	30.8	2.0	1.1	—	45.3	3.2	2.9	—	0.0	0.0	0.0	—
F. diversisporium	1	2+	7.8	4.2	1.4	—	23.3	3.0	2.3	—	0.0	0.0	0.0	—
F. nivale	1	3+	6.5	31.4	7.7	—	16.5	31.4	2.6	—	0.0	0.0	0.0	—
F. tumidum	1	2+	2.5	2.7	2.1	—	15.5	6.8	3.4	—	0.0	0.0	0.0	—
Hormodendrum														
H. nigrescens	2	3+	30.3	63.5	1.0	78.8	43.3	67.3	2.3	71.7	0.0	0.0	0.0	0.3
Monilia														
M. brunnea	1	3+	22.4	56.3	4.5	79.1	8.2	15.6	1.0	20.7	0.0	0.0	0.0	0.0
Penicillium														
P. aurifluum	2	3+	8.0	42.0	5.7	—	17.1	50.8	5.3	—	0.0	0.0	0.0	—
P. casei	2	3+	66.4	94.8	3.9	86.0	80.4	91.7	3.3	79.3	0.0	0.0	0.0	0.0
P. gilmanii	2	3+	17.8	46.3	1.0	—	24.1	64.9	4.0	—	0.0	0.0	0.0	—
P. miczynskii	1	3+	18.6	5.6	1.0	—	21.5	29.7	8.0	—	0.0	0.0	0.0	—
P. oxalicum	1	3+	48.7	75.4	1.9	—	72.5	83.4	17.7	—	0.0	0.0	0.0	—
P. puberlum	1	3+	74.5	81.1	1.0	—	82.4	82.3	2.7	—	0.0	0.0	0.0	—
P. roqueforti	1	3+	48.4	80.6	1.0	—	66.2	74.1	2.8	—	0.0	0.0	0.0	—
P. stoloniferum	1	3+	44.6	64.3	—	—	52.4	72.8	—	—	0.0	0.0	0.0	—
P. sauvolens	1	3+	24.0	61.8	3.8	—	35.4	69.3	3.6	—	0.0	0.0	0.0	—
P. trzebinskii	1	3+	49.8	81.6	3.1	—	47.1	53.7	3.1	—	0.0	0.0	0.0	—
Pullularia														
P. pullulans	6	2+	10.9	18.7	3.2	95.2	22.4	18.6	3.1	51.5	0.0	0.0	0.0	0.1
Hormiscium														
H. species	1	2+	45.5	58.6	1.6	48.7	32.0	48.8	2.0	19.7	0.0	0.0	0.0	0.1
BACTERIA														
Gram positive, sporeforming rods, aerobic (*Bacillus*)	11	4+	2.7††	2.3	2.6	3.4	6.3	4.2	2.6	3.6	0.0	0.0	0.0	0.0
			0.0	0.0	0.0	1.7	0.0	0.0	1.0	1.7				
	39	3+	4.8	4.8	—	—	6.9	6.8	—	—	0.0	0.0	—	—
			0.0	0.0			0.0	0.0						
	27	2+	8.8	—	—	3.2	6.2	—	—	1.4	0.0	—	—	0.0
			0.0			0.0	0.0			0.0				
	22	+	4.1	—	—	5.5	4.2	—	—	1.3	0.0	—	—	0.0
			0.0			0.0	0.0			0.0				
	51	0	3.4	4.0	—	4.8	5.1	7.1	—	1.2	0.0	0.0	—	0.0
			0.0	0.0		0.0	0.0	0.0		0.0				
YEASTS														
Unidentified	15	+	2.8	6.2	1.6	4.4	1.0	10.2	3.6	1.0	0.0	0.0	0.0	0.0
			0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0				

* Sodium polypectate gel.
† The action of microbial enzymes on a 0.75 per cent sodium polypectate solution at pH 5.3 after 20 hr at 30 C.
‡ The action of microbial enzymes on a 1.1 per cent pectin (8.0 per cent methoxyl) solution at pH 5.3 after 68 hr at 30 C.
§ The action of microbial enzymes on a 1.0 per cent pectin (8.0 per cent methoxyl) solution after 68 hr at 30 C.
¶ 4+ = Very strongly active; 3+ = strongly active; 2+ = moderately active; + = weakly active; 0 = nonpectolytic.
‖ All values corrected by controlled readings.
** No test.
†† Values form the activity range (highest and lowest) for the group.

The action of the enzymes elaborated by the various organisms is given in table 1. The values given for the fungi enzyme tests are averaged values from at least two readings, while the values from the bacterial and yeast tests form the range of values (highest and lowest) for the particular groups.

Discussion

The use of a rapid screening medium, such as the sodium polypectate gel, for the detection of pectolytic organisms has a number of drawbacks in a study of microorganisms capable of salt-stock softening. The first and most important of these is the failure of this type of test to indicate whether organisms isolated have the ability to hydrolyze pectic substances under the conditions of normal fermentation. Another problem with such a procedure is the specificity of the medium. The compound, sodium polypectate, is degraded by enzymes of the polygalacturonase group and an organism hydrolyzing the glycosidic linkages of this compound might be unable to attack pectin or parent protopectin of the cucumber because of the absence of the de-esterifying enzyme, pectinesterase. Polymethylgalacturonase will, of course, degrade pectin without this enzyme (Demain and Phaff, 1957). Thus, an organism giving positive results for pectolytic activity on this medium would not necessarily be responsible for cucumber softening under commercial conditions.

The approximate method of enumeration employed in this work indicates that the fungal populations of the commercial vats tested were very low in comparison to other types of organisms. Only 15 filamentous fungi were obtained in 1958 and 41 in 1959 from brines of the test vats and there appears to be a relationship between the numbers observed and the lack of softening (Hamilton and Johnston, 1961).

The results of the simulated brine tests show that the bacteria and yeasts, although pectolytic on the sodium polypectate gel, were unable to produce any type of activity under the experimental conditions. This lack of pectolytic activity included the control series (test IV) which would appear to indicate that the enzymes of these cultures were not specific for pectin and require a lower polyuronide before action can be detected.

All the readings obtained from the bacterial and yeast tests were negative for activity under the conditions of the experiment and the lack of pectinesterase activity by the yeast is surprising in view of the results obtained by Etchells and Bell (1950) and Bell and Etchells (1956) indicating the de-esterification of pectin by both surface and subsurface cultures. Thus, it would appear that yeasts are not highly active under commercial conditions with respect to pectinesterase elaboration.

The fungi, on the other hand, were found to be much more active under the experimental conditions. Although the enzyme tests were organized on a qualitative basis, some evidence of a quantitative nature can be gained from the data despite a crude method of inoculation. All the values for the fungi were obtained in duplicate, and in some cases, the readings obtained were the average of as many as 14 tests with the same species. No comparison with previous work by Etchells and co-workers (1958) is possible as the values obtained in that work were based on the activity of fungal filtrates after 14 days growth as compared to 3 days growth in this work.

Test IV, the control series, indicated that the fungi were capable of producing pectolytic enzymes in the presence of pectin. No effort was made to determine whether pectin was required for the formation of these enzymes (adaptive) or that they were formed regardless of the substrate (constitutive).

With reference to the polygalacturonase test, the results indicate that the majority of the filamentous fungi produced an enzyme or a group of enzymes which resemble polygalacturonase by their action on sodium polypectate. With the exception of species of *Cephalosporium*, *Fusarium*, and *Pullularia*, the results indicate that the majority of the test fungi were at least moderately active under the experimental conditions. In addition, most of the readings show that the addition of 6.0 per cent salt in test I was detrimental to the elaboration of this enzyme when compared to the control (test IV) results.

Species of *Alternaria* were found to be moderately active at pH 5.3 and 6.0 per cent salt. The activity of this group was severely curtailed at pH 4.0 and 9.0 per cent sodium chloride (test II) and inactive when grown at pH 3.4 and 12.8 per cent salt.

The *Aspergillus* group was more variable. *Aspergillus flavus* showed negligible to weak activity at the lower concentrations, although some increase was noticed in test II. On the other hand, *Aspergillus niger* showed very high activity in all three tests. In fact, as the salt and acid were increased the enzyme activity appeared to increase in a similar manner. *Aspergillus sydowi* was shown to increase at higher concentrations also, while *Aspergillus fumigatus* decreased in activity with increases in salt and acid.

Of the three species of *Cephalosporium* tested, only *Cephalosporium roseo-griseum* was found to show moderate activity in tests I and II followed by negligible readings at pH 3.4 and 12.8 per cent salt.

The genera, *Epicoccum* and *Pullularia*, showed moderate to weak activity under the simulated vat conditions despite very high readings in the control tests.

With the exception of *Fusarium culmorum* and *Fusarium nivale*, the genus *Fusarium* showed very little activity and would appear to be a poor source of polygalacturonase activity under vat conditions. However. Etchells and co-workers (1958) found that this

genus was one of the most frequently isolated of all the fungi they studied. In addition, they considered it to be a highly important source of pectolytic enzyme activity.

The genera, *Hormodendrum*, *Monilia*, and *Hormiscium*, were shown to produce more activity at pH 4.0 and 9.0 per cent salt than in test I and although they were moderate to strongly active in test II their activity was severely limited in test III.

Species of *Penicillium* generally were found to be highly pectolytic and only *Penicillium miczynskii* demonstrated weak to negative activity. Here again, the activity of this group increased at pH 4.0 and 9.0% salt followed by negligible results in test III.

Of all the fungi tested only *A. niger* and to a lesser extent *A. sydowi* were found to withstand the extreme environmental conditions of test III.

The polymethylgalacturonase enzyme test gave results similar to the PG tests, although no comparison is possible because of the faster action of the polygalacturonases. The pectolytic enzymes produced by the fungi were able to degrade pectin without the aid of pectinesterase as shown by the negative results in the PE test series. Thus, the fungi appear to elaborate either two separate enzyme systems for the hydrolysis of pectin and pectic acid, or one enzyme system capable of attacking both substrates. It has been generally concluded, however, that filamentous fungi do elaborate a number of pectolytic enzymes and it is possible that action detected in the PG tests and PMG tests were the results of at least two separate enzymes (Demain and Phaff, 1957).

In summary, it can be concluded that species of *Alternaria*, *Aspergillus*, *Hormodendrum*, and *Penicillium* are potent sources of pectolytic enzymes and could be implicated in cucumber softening. The softening of cucumbers by these genera would, of course, depend on the initial population present in the brine and the rate of pectolytic enzyme production.

It is obvious from this work that the bacteria and yeasts normally found in cucumber salt-stock brines were not sources of pectolytic enzymes under simulated vat conditions. This information, added to the long list of similar evidence by other workers, would appear to eliminate the bacteria and yeasts as sources of pectolytic softening of cucumbers under commercial salt-stock conditions.

On the other hand, the filamentous fungi isolated from the salt-stock brines were found to elaborate enzymes similar to polygalacturonase and polymethylgalacturonase, but were not observed to produce pectinesterase. Thus, the fungi are logical sources of pectolytic softening, but more work would have to be done to establish the numbers required and the environmental conditions promoting softening under commercial vat conditions. Also, the possibility exists that the cucumber and its accessory parts are sources of pectolytic enzymes. In this regard, it might be deduced, since none of the pure cultures studied elaborated pectinesterase and since this enzyme was detected in the commercial brines (Hamilton and Johnston, 1961), that the cucumber is responsible for the elaboration of this enzyme. This would confirm the opinion of Bell, Etchells, and Jones (1951), who found the cucumber to be a source of pectinesterase. Further, the results of the commercial brine studies indicate that the pectinesterase activity increases with a decrease in the size of the cucumber, which would appear to confirm the implication of the cucumber in this respect. It is also possible that some polygalacturonase activity of commercial brines might originate with the cucumber as high PG readings were detected with the small sized cucumbers (Hamilton and Johnston, 1961).

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Summary

Ninety-nine gram positive, sporeforming bacteria, presumably belonging to the genus *Bacillus*, and 15 yeast cultures, isolated from commercial cucumber salt-stock brines, were unable to produce pectolytic enzymes under simulated commercial conditions, although they had the ability to hydrolyze sodium polypectate gel.

Species belonging to 10 genera of filamentous fungi were found to be moderate to highly pectolytic when tested under the same conditions. The fungi produced enzymes resembling polygalacturonase and polymethylgalacturonase, but did not produce pectinesterase.

Reference was made to the incidence of pectinesterase in commercial vats, although the enzyme was not detected in the pure culture study. This would seem to implicate the cucumber as the source of this enzyme. It was concluded that both the cucumber and filamentous fungi could be responsible for pectolytic softening under commercial salt-stock conditions.

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Scale-up of Heat Sterilization Operations[1]

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Sterilization of a fermentation medium is usually accomplished in a cycle that includes heating the medium to some prescribed so-called sterilization temperature, holding it there for a specified period of time, and cooling it to process temperature. The productive capacity of most media is adversely affected by prolonged heating due to degradation of some nutrients or the production of substances which are toxic to the microorganisms used in the subsequent fermentations. In many cases, therefore, media sterilization presents two incompatible objectives. On the one hand, it is desired to maximize contaminant destruction, while on the other, damage to the nutrient quality of the medium must be minimized.

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Although continuous sterilization offers inherent advantages with regard to the objectives of a sterilization operation, very little work has been published describing in just what manner these objectives can be optimized, or even translated in process scale-up. Design is based largely on achieving satisfactory contaminant destruction with little concern other than one of conveniently trying to minimize the heat exposure. Contaminant destruction calculations can be carried out quantitatively but damage to nutrient quality has usually been examined only in a qualitative light. The same remarks, of course, also apply to batch sterilization.

This paper briefly examines quantitative changes in both contaminant destruction and damage to nutrient quality in a medium as scale of operation is changed. A quantitative basis for nutrient quality damage rests on the fact that adverse changes which occur in the medium during sterilization are chemical reactions